LANSCE DIVISION TECHNOLOGY REVIEW

Protein Crystallography Station—Commissioned and Ready for Users

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The new neutron Protein Crystallography Station (PCS)[1] located at the Los Alamos Neutron Science Center (LANSCE) was declared "ready for beam" in December 2000. In 2001, the PCS was fully commissioned by B Division with support from LANSCE. The PCS, which is funded by the Department of Energy Office of Biological and Environmental Research, will become part of the LANSCE User Program in 2002. The PCS is located at the Lujan Neutron Scattering Center (Lujan Center) on flight path 15, viewing a coupled water moderator where neutrons are emitted in pulses at a rate of 20 or 30 Hz.

System Development and Installation

The neutron beam transport system and shielding were installed before the end of the 2000 LANSCE run cycle. The neutron beam transport system consists of about 28 m of vacuum pipe between the neutron source and the sample position as shown in Fig. 1. The vacuum pipe carries the neutrons out of experimental area ER-1 through a wall into ER-2 and has collimation inserts that taper the incident neutrons to produce a fine, almost parallel beam that hits the crystal sample. These inserts extend back into bulk shielding that surrounds the moderator where a 2-m section of beam pipe can be filled with mercury to act as a shutter for opening and closing the neutron beam. The vacuum pipe is mounted on adjustable stands and was aligned optically to survey monuments (cross marks) that were laid when the mercury shutter was installed in the bulk shield in 1999.

A shielding of iron and polyethylene laminate was installed around the vacuum pipe in ER-1 with provisions for a cavity for the future installation of a chopper. A magnetite concrete and polyethylene shield was installed around the vacuum pipe in ER-2.

The shielding opens up to a large cave at the sample position in ER-2. This cave was installed as interlocking monolithic blocks that were bolted together. After the shutter and personal access control systems were installed and tested and after a background radiation survey was completed, the station was declared "ready for beam" with two weeks of beam time left in the 2000 run cycle.

Receiving first beam at the end of the 2000 run cycle was a tremendous achievement for which the installation team (LANSCE-7) and the ER-1 shielding designer (Kathy Lovell, LANSCE-12) received Distinquished Performance Awards. These two weeks of beam time proved to be extremely important. In our first experiment, line shapes of 9 orders of diffraction data were collected from a fluorophlogopite crystal to characterize, for the first time, the neutron time dispersion of the coupled water moderator (Fig. 2).[2] The results

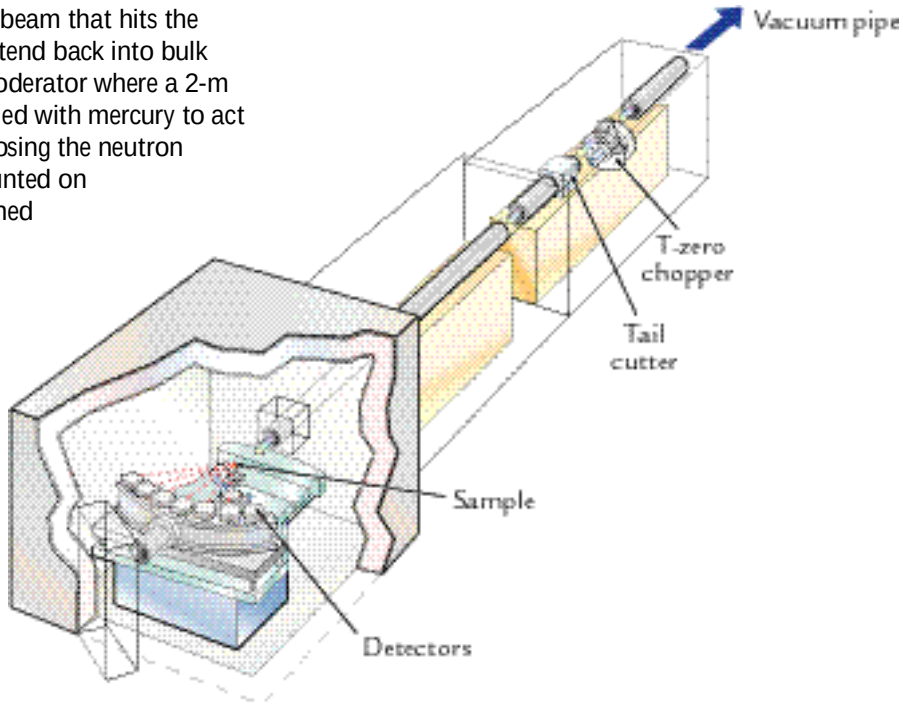
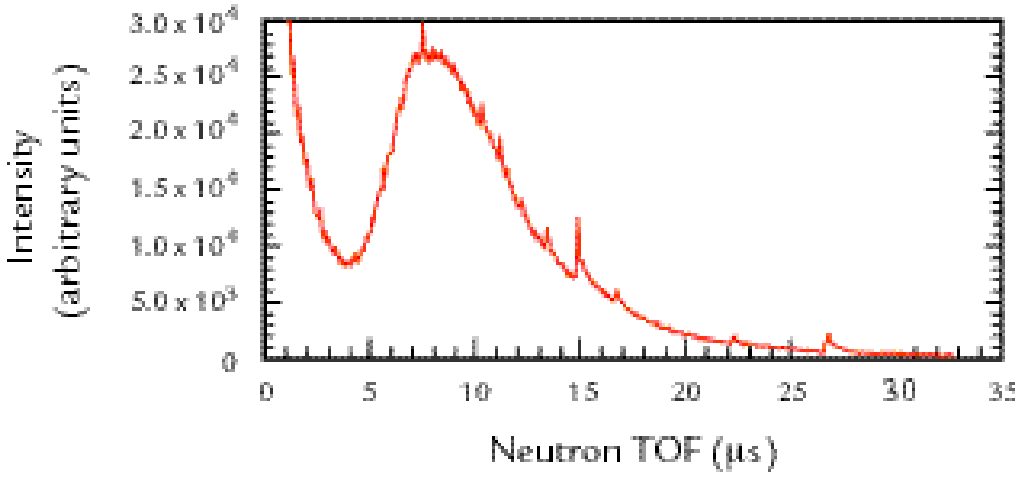


Fig. 1. *Protein Crystallography Station beam layout.*



↑ ***Fig. 2.*** *A time-of-flight (TOF) diffraction pattern from a fluorophlogopite crystal. The various Bragg peaks correspond to wavelengths ranging approximately from 1 Å to 4 Å.*

indicate that the "coupling" of the spallation neutrons to the moderator is smaller than projected, implying a shortfall in neutron flux but a better time resolution. Because the measured, integrated neutron flux of the direct beam at the sample position was a factor of 40 below projections, the neutron beam transport system was judged to be neutronically misaligned.

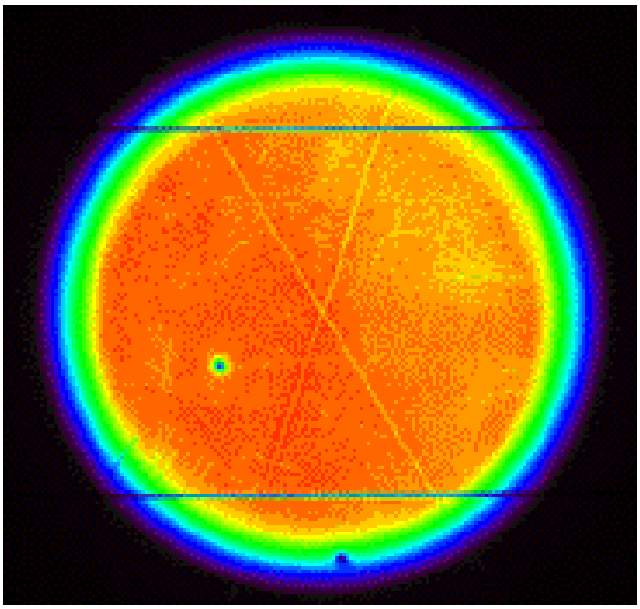
Data were successfully collected from a Vd-Nb sample using a small 200-mm x 200-mm position-sensitive detector provided by Brookhaven National Laboratory (BNL) and an initial VME (virtual memory extension) single-crate version of the data-acquisition system (DAQ).

Beam Realignment

During the scheduled outage in 2001, the shielding was disassembled and the optical alignment of the neutron beam transport system was found to be consistent with the survey monuments. When neutrons became available again in June 2001, the easily accessible part of the vacuum pipe was realigned to follow the peak in the distribution of the neutron beam flux. We determined this distribution by placing neutron-image-plate detectors and pinhole collimators at various positions along the beam line (Fig. 3). This alignment of the neutron beam differed by 0.01° from the optical alignment and improved the direct-beam-integrated neutron beam flux at the sample position by more than a factor of 10. The neutron beam flux remains a factor of 3 to 4 smaller than projected, and we are still investigating whether this finding is due to moderator performance or beam misalignment before the chopper cavity.

Final Installations

During August 2001, a team from BNL replaced the small detector and its electronics with a larger 120° x 16° detection system. Shortly after installation, a number of small problems with the detector and its communication with the DAQ were identified and have since been rectified. The detector and a VME multi-crate version of the DAQ were commissioned, and an instrument control system was developed andinstalled. The instrument control system



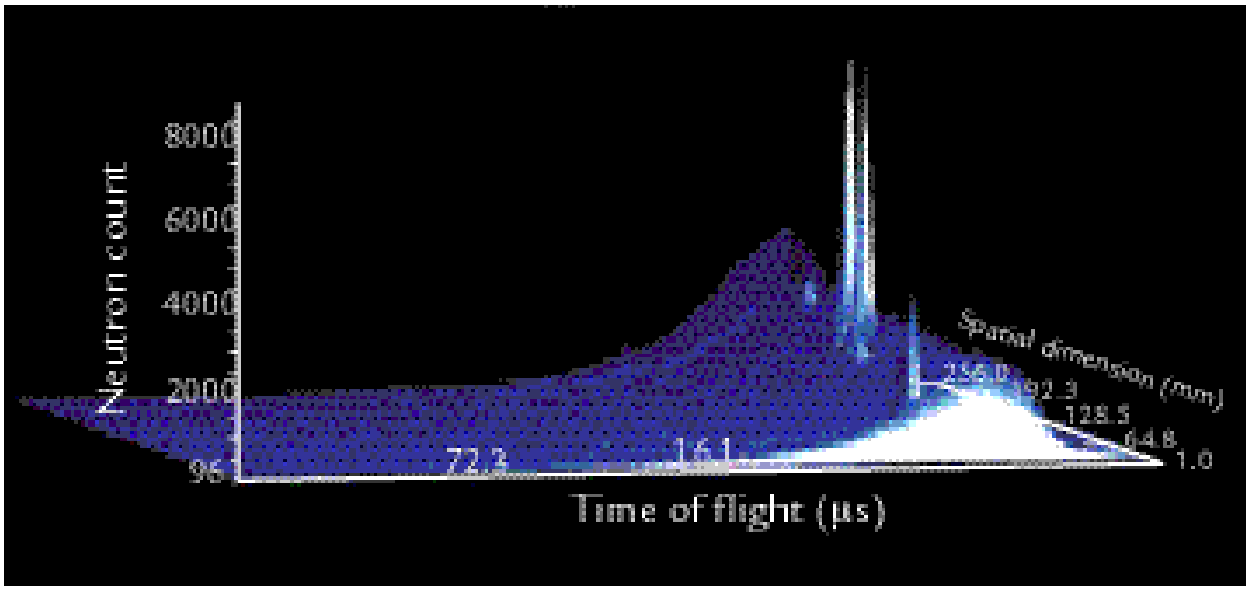
↑ ***Fig. 3.*** *A neutron-image-plate recording of the neutron beam flux. The image plate was placed at a break in the vacuum beam pipe, 11 m from the moderator, with cross wires marking the center of the vacuum pipe. The recorded neutron flux increases from blue to red. This image shows that the beam has a circular profile and is fairly homogeneous.*

controls the sample and detector goniometer motors and interfaces with the DAQ to coordinate data collection with motor movements. A graphical user interface, which is unique to PCS, provides a user-friendly means of conducting experiments. The station was finally ready for commissioning experiments after the chopper was successfully installed and tested in ER-1 by September 2001.

Commissioning Experiments

Commissioning experiments were chosen to test various aspects of the PCS. Single crystals of myoglobin, cobalamine (II), and α-glycine were used to probe the diffraction resolution of the PCS and also to provide data sets for testing the data-analysis software. Myoglobin is an oxygen-transport protein with a relatively large unit cell that diffracts neutrons to about a 2-Å resolution. Cobalamine (II) is a reduced form of the vitamin B12 coenzyme, and α-glycine is an amino acid and neurotransmitter. Both have smaller unit cells and diffract neutrons to atomic resolution. Complete data sets were collected from all three systems. Data were collected well beyond the projected 1.5-Å resolution limit of PCS (Fig. 4). To test the performance of the PCS for fibrous samples, we also collected complete data sets from the polysaccharide cellulose Iβ, which was extracted from the mantles of *Halocynthia roretzi*,[3] and from calf thymus DNA.

In the final two weeks of the 2001 run cycle, we tested the feasibility of future experiments with samples supplied by users. These samples included single crystals of insulin grown for microgravity experiments in space;[4] the photosynthetic reaction center (PRC) of *Rhodobacter sphaeroides*, which is a large membrane-bound protein complex responsible for converting photons of light into chemical energy;[5] and oligonucleotide d(CGCGCG), which is a short synthetic fragment of DNA. The most striking results were obtained with the PRC of *Rhodobacter sphaeroides*, whereby a crystal was placed in the beam for about a fifth of the normal exposure duration with a reduced proton beam delivery of 48 μA rather than at the 200-μA design specification. The crystal had a volume below the lower end of the design feasibility range for the PCS (< 1 mm^3) and a unit-cell volume three times larger than the upper end of the design feasibility range for the PCS (i.e., 1 x 10^6 $Å^3$). Despite these circumstances, diffraction data (collected to < 5-Å resolution) indicate that under normal operations data taken at much higher resolution could be collected on the PCS from this large protein complex (Fig. 5).



↑ *Fig. 4.* *Diffraction data collected from cobalamine (II) in a small area of the detector. The data, which were collected in two spatial dimensions and one time dimension, have been summed along one of the spatial dimensions so that it can be represented as an isometric view with the TOF axis in the horizontal position and the remaining spatial axis pointing into the page. The slowly varying background scattering is due mostly to unwanted incoherent scattering from hydrogen and reflects the distribution of neutron wavelengths in the incident-beam spectrum. The sharp peaks that sit on the background are the desired Bragg diffraction peaks from the crystal.*

Conclusion

The PCS has been commissioned and is ready to become part of the LANSCE User Program in 2002.

Experiments carried out during the commissioning period are encouraging and indicate that the PCS will become a world-class, state-of-the-art instrument for structural biology.

References

1. P. Langan and B.P. Schoenborn, "Protein Crystallography Station—Solving Protein Structures with Innovative Time-of-Flight Neutron Diffraction Techniques," Los Alamos National Laboratory report LALP-01-183.
2. Measurements courtesy of L. Daemen, LANSCE-12.
3. Sample provided by Y. Nishiyama, Tokyo University.
4. Sample provided by R. Blessings, State University of New York.
5. Sample provided by M. Yousef, P. Thiyagarajan, and P. Laible, Argonne National Laboratory.

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